P-69E

Proteomics Analysis for Off-Target Risk Assessment of Targeted Protein Degraders

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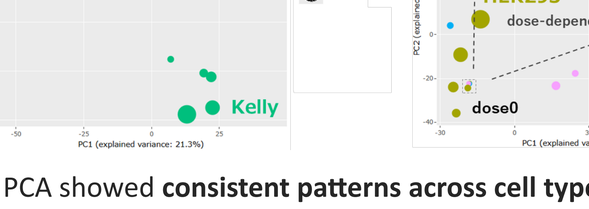
Background

Modality-specific safety risks of TPDs (targeted protein degraders) :
Not entirely selective for the intended target
→ **Off-target degradation**

Mechanism of degradation by TPD



Ub: Ubiquitin
POI: Protein of Interest

Pain points (key issues)	Our solutions
• **Animal species differences** in TPDs → Limited prediction of human toxicity based on animal studies • Difficult-to-assess toxicities during clinical trials (e.g., carcinogenicity, teratogenicity) → Late-stage risk	**Proteomics in human-derived cells** → Human-relevant *in vitro* off-target evaluation at an early-stage
• Safety-critical proteins (e.g., SALL4) may be **detectable only in specific cell types**[1] → Easily missed • Low expression of safety-critical proteins → Low-abundance off-targets are difficult to detect	**Deep, quantitative global proteomics of a multi-cell-type panel** → Broad coverage of known safety-risk neo-substrates
• Not all off-target degradations imply equal risk → Requires expert interpretation	**Toxicity panel assessment** based on **toxicity-associated phenotypes** → Clear safety profiles for feedback to medicinal chemistry & decision-making

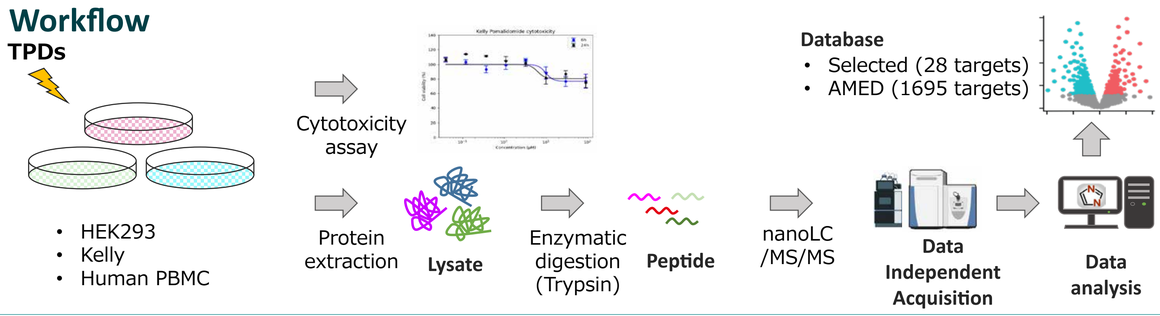
Key Strengths of Our TPD Safety Evaluation

- Proteome-wide evaluation enables comprehensive TPD off-target profiling
- A multi-cell-type panel ensures broad, human-relevant proteome coverage (approximately 10,000 proteins)
- Integrated safety annotation enables efficient risk interpretation and prioritization

Objective

To establish and validate a proteomics-based workflow for TPD off-target safety profiling using a multi-cell-type panel.

Workflow

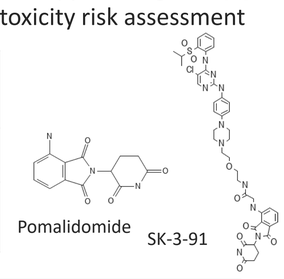


Methods & Materials

- Global proteomics of HEK293, Kelly, and hPBMC treated with pomalidomide or SK-3-91
- The multi-cell-type panel was selected based on coverage of 28 known high-risk proteins, including known IMiDs (Immunomodulatory Drugs) or other TPD substrates [2,3,4,5], potential substrates [5], tumor suppressor genes [6]
- Use of the AMED off-target risk list [7] and HPO (Human Phenotype Ontology) analysis for toxicity risk assessment

Compounds	Types	Primary targets	E3 ligase	Cells	Treatment concentration (μM)	DMSO concentration (% v/v)	Time (h)
Pomalidomide	IMiD* molecular glue	IKZF1, 3	CRBN	• **HEK293** (ATCC: CRL-1573) • **Kelly** (Cytion: 300317) • **hPBMC** (Charles River: PB009C-1)	0, 0.12, 0.37, 1.1, 3.3, 10	0.1% v/v	6
SK-3-91	PROTAC	Multiple kinases	CRBN		0, 0.04, 0.12, 0.37, 1.1, 3.3	0.033% v/v	6

*IMiD: Immunomodulatory Drug; representative examples include thalidomide and its derivatives

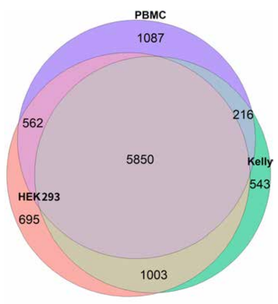


Results & Discussion

Figure 1. PCA (Principal Component Analysis) of global proteome profiles

✓ PCA showed **consistent patterns across cell types and concentrations**, supporting the overall quality of the dataset.

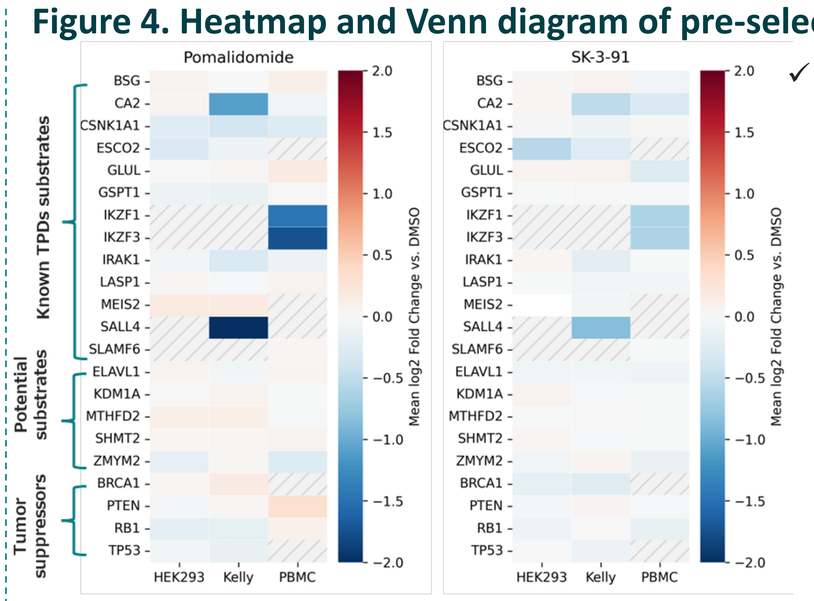
Figure 2. Venn diagram of identified proteins



9,956 proteins

✓ The multi-cell-type panel enables **broad proteome coverage**.

Figure 4. Heatmap and Venn diagram of pre-selected candidates



✓ The multi-cell-type panel enabled detection of 22 of 28 pre-selected safety-relevant proteins, **enhancing the detection coverage of known IMiD neo-substrates**, such as SALL4 and IKZF1/3.

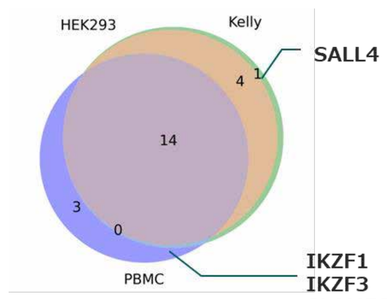
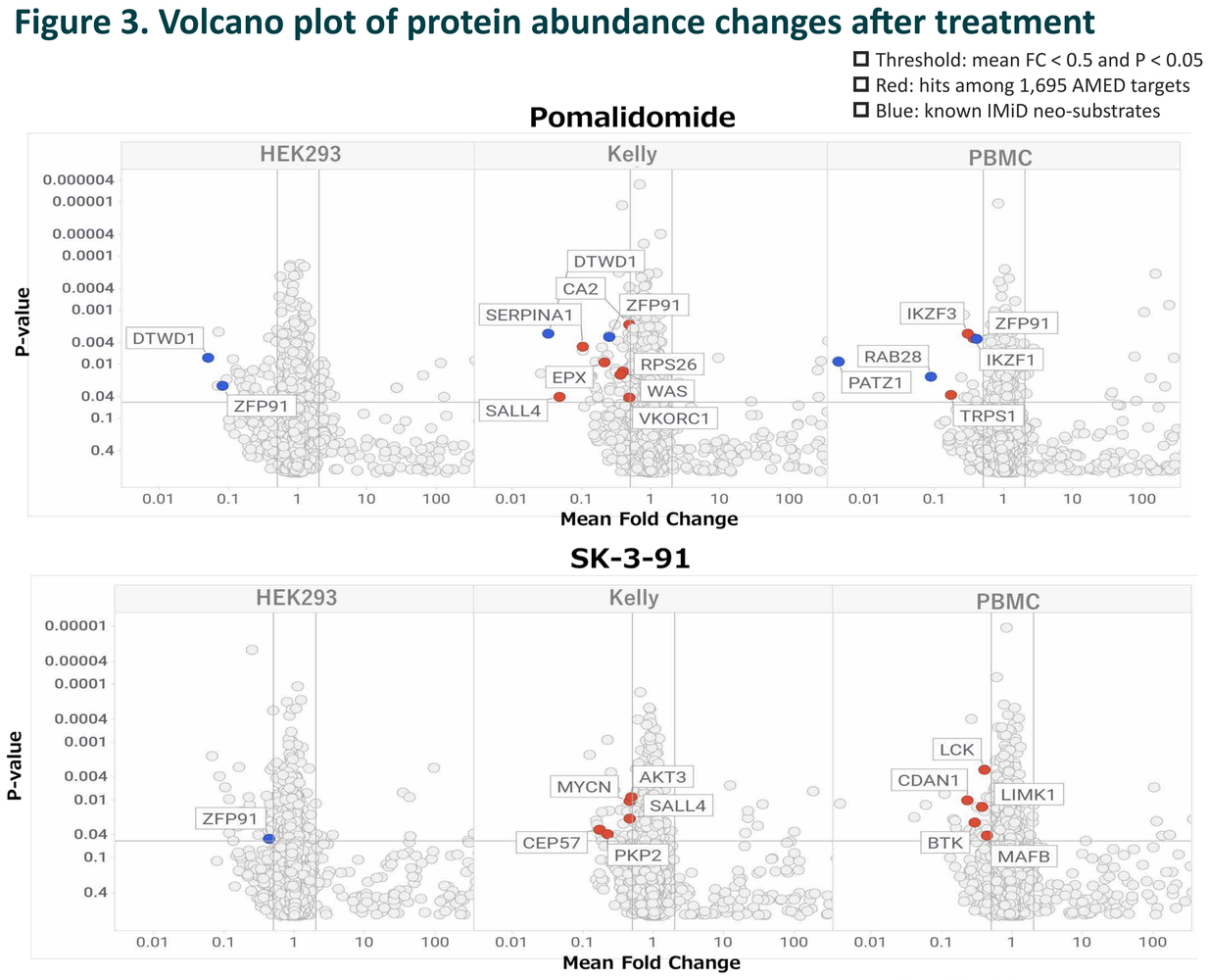


Figure 3. Volcano plot of protein abundance changes after treatment

- Threshold: mean FC < 0.5 and P < 0.05
- Red: hits among 1,695 AMED targets
- Blue: known IMiD neo-substrates



✓ **Changes in safety-relevant targets were highlighted** within broad proteomic fluctuations.

✓ Dose-response analysis helped distinguish likely **true degradation events** in a dose-dependent manner, as shown by the pomalidomide examples.

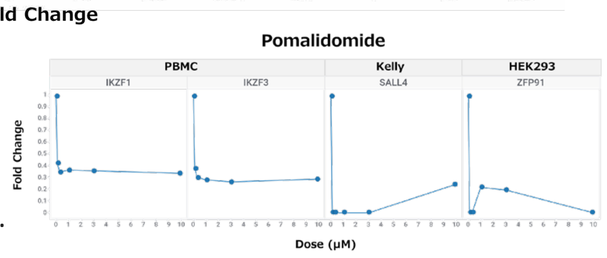
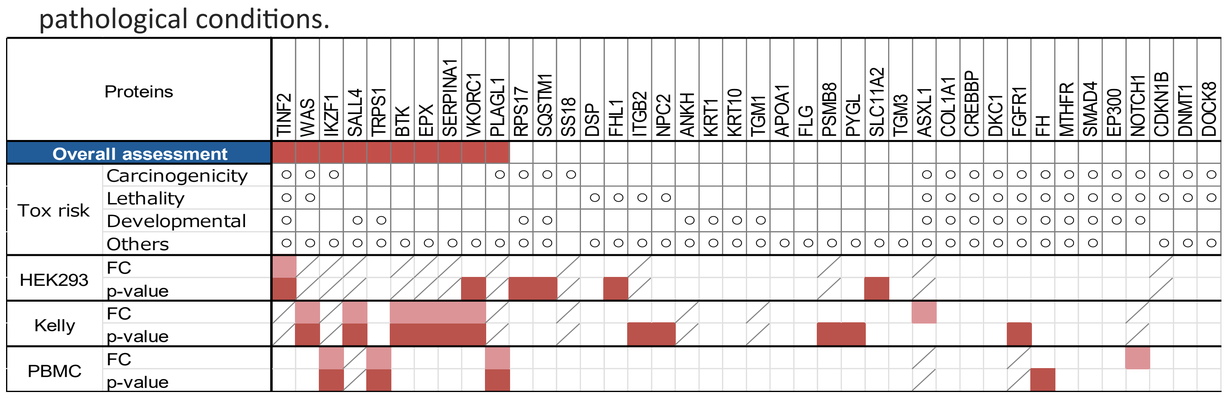


Figure 5. Toxicity panel assessment: Case study of pomalidomide

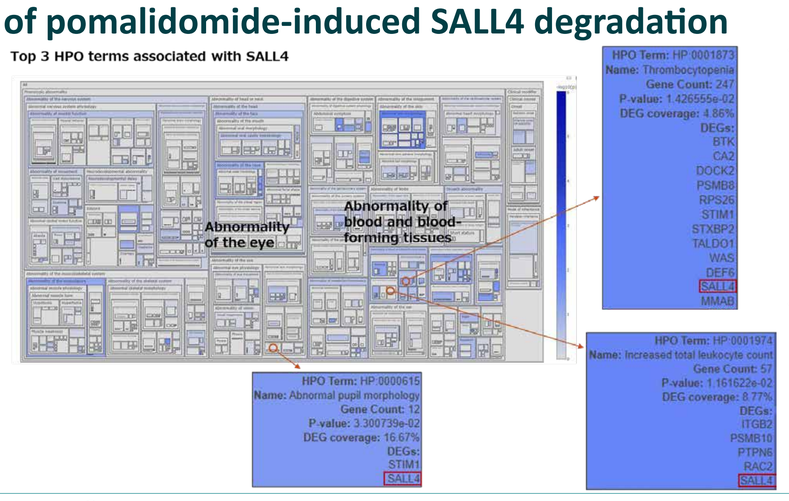
✓ Our proteomics dataset **covered 989 of 1,695 genes** in the **AMED off-target risk assessment list** [7], curated from human genetic disease and mouse knockout phenotype data.

✓ Cross-referencing with the AMED list enabled interpretable **assessment of potential safety liabilities, such as** carcinogenicity, lethality, developmental abnormalities, and other pathological conditions.



☐ Top 40 genes are shown
☐ Overall assessment: red: hits; circles: toxicity associations
☐ Light red: FC (fold change) < 0.5; dark red: significant hits with P < 0.05
☐ Diagonal lines: undetectable in this cell type, blank columns: no change

Figure 6. HPO (Human Phenotype Ontology) enrichment analysis: Case study of pomalidomide-induced SALL4 degradation



✓ **Example:** The top 3 HPO terms associated with SALL4 related to eye and blood-forming tissues, suggesting potential developmental and hematopoietic safety liabilities.

✓ HPO and pathway enrichment support **functional interpretation** and **safety assessment of proteins with unclear toxicity**.

Summary

- ☐ Multi-cell-type panel proteomics enables broad and human-relevant off-target detection.
- ☐ Integration with toxicity annotations enables interpretable safety assessment.
- ☐ This approach supports the selection of safer and more selective TPD candidates.

References

[1] Garside et al. Nat. Rev. Drug Discov. (2026).
[2] Donovan et al. eLife 7:e38430 (2018).
[3] Federspiel et al. Mol. Cell. Proteomics 23:100797 (2024).
[4] An et al. Nat. Commun. 8:15398 (2017).
[5] Baek et al. Nat. Commun. 16:6831 (2025).
[6]大岡伸通. AMED「創薬基盤推進研究事業」研究成果発表会 (2020).
[7]AMED.「核酸医薬品のオフターゲット効果のリスク評価に資するヒト遺伝子機能の抽出と分類に関する調査 (https://www.amed.go.jp/program/list/17/01/yakuzai_kakusaniyakuhin.html) Accessed March 2026.

COI

Lead Presenter/Responsible Researcher: **Qinge LIANG**
I have no COI to disclose.

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